

University of Groningen

**Long-term effects of husbandry procedures on stress-related parameters in male mice of two strains**

van Loo, P.L.P.; van der Meer, E.; Kruitwagen, C.L.J.J.; Koolhaas, J.M.; van Zutphen, L.F.M.; Baumans, V.

*Published in:*  
Laboratory Animals

*DOI:*  
[10.1258/002367704322968858](https://doi.org/10.1258/002367704322968858)

**IMPORTANT NOTE:** You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

*Document Version*  
Publisher's PDF, also known as Version of record

*Publication date:*  
2004

[Link to publication in University of Groningen/UMCG research database](#)

*Citation for published version (APA):*

van Loo, P. L. P., van der Meer, E., Kruitwagen, C. L. J. J., Koolhaas, J. M., van Zutphen, L. F. M., & Baumans, V. (2004). Long-term effects of husbandry procedures on stress-related parameters in male mice of two strains. *Laboratory Animals*, 38(2), 169-177. <https://doi.org/10.1258/002367704322968858>

**Copyright**

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

**Take-down policy**

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

---

# Long-term effects of husbandry procedures on stress-related parameters in male mice of two strains

P. L. P. Van Loo<sup>1</sup>, E. Van der Meer<sup>1</sup>, C. L. J. J. Kruitwagen<sup>2</sup>,  
J. M. Koolhaas<sup>3</sup>, L. F. M. Van Zutphen<sup>1</sup> & V. Baumans<sup>1,4</sup>

<sup>1</sup>Department of Laboratory Animal Science, Utrecht University, PO Box 80.166, 3508 TD Utrecht, The Netherlands, <sup>2</sup>Center for Biostatistics, Utrecht University, Padualaan 14, 3584 CH Utrecht, The Netherlands, <sup>3</sup>Department of Animal Physiology, University of Groningen, PO Box 14, 9750 AA, Haren, The Netherlands and <sup>4</sup>Karolinska Institutet, 17177 Stockholm, Sweden

## Summary

In socially unstable groups of male laboratory mice, individuals may experience a chronic stress situation. Previous experiments have shown that the transfer of specific olfactory cues during cage cleaning, and the provision of nesting material decrease aggression and stress in group-housed male mice. In this study, the combined effect of these husbandry procedures were tested for their long-term effect on stress in groups of moderately aggressive (BALB/c) and severely aggressive (CD-1) male mice. The physiological and behavioural stress-related parameters used were body weight, food and water intake, spleen and thymus weight, adrenal tyrosine hydroxylase activity, urine corticosterone levels and behaviour in a cage emergence test. Long-term provision of nesting material and its transfer during cage cleaning was found to influence several stress-related physiological parameters. Mice housed in cages enriched with nesting material had lower urine corticosterone levels and heavier thymuses, and they consumed less food and water than standard-housed mice. Furthermore, marked differences were found between strains. CD-1 mice were less anxious in the cage emergence test, weighed more, ate and drank more, and had heavier thymuses but lighter spleens and lower corticosterone levels than BALB/c mice. We conclude that the long-term provision of nesting material, including the transfer of nesting material during cage cleaning, reduces stress and thereby enhances the welfare of laboratory mice.

**Keywords** Husbandry; environmental enrichment; male mice; stress; welfare

Laboratory mice are generally housed in confined, stimulus-poor environments in which the performance of natural behaviour is impaired. As a result, the animals may show abnormal behaviour such as stereotypies and apathy (Chamove 1989, Cooper & Nicol 1996, Würbel *et al.* 1998). When male mice are housed in groups, their inability to perform normal social behaviour may

additionally trigger excessive aggression and, as such lead to a situation of chronic social stress. The use of environmental enrichment to improve the well-being of laboratory mice is promoted widely and is recently incorporated in European legislation (Council of Europe 1997). Its general aim is to enhance species-specific behaviour, promote physical health as far as possible and to decrease abnormal behaviour while keeping a focus on scientific, economic and ergonomic demands (Newberry 1995, Dean 1999,

Correspondence to: P. L. P. Van Loo  
E-mail: P.L.P.vanloo@vet.uu.nl

Baumans 2000). Before applying any kind of environmental enrichment, its presumed effect on the well-being of the animals, both in the short and in the long term, need to be verified (Shepherdson *et al.* 1998). Several kinds of environmental enrichment have been shown to decrease inter-male aggression (Ward *et al.* 1991, Armstrong *et al.* 1998, Ambrose & Morton 2000), whereas other enrichments appear not to affect inter-male aggression (Eskola & Kaliste-Korhonen 1999), or even increase inter-male aggression (McGregor & Ayling 1990, McGregor *et al.* 1991, Haemisch & Gärtner 1994, Haemisch *et al.* 1994, Van Loo *et al.* 2002). In previous experiments we found that the amount of aggression in group-housed male mice significantly decreased when nesting material was introduced in the cages (Van Loo *et al.* 2002), and when nesting material was transferred during cage cleaning (Van Loo *et al.* 2000). These studies were carried out with an inbred strain (BALB/c), known to be moderately aggressive (Eskola & Kaliste-Korhonen 1999, Van Loo *et al.* 2000) and particularly susceptible to chronic stress exposure (Kopp *et al.* 1999).

The aim of the present study was to investigate the long-term effect on aggression and stress-related parameters of a combination of factors, previously found to decrease aggression in group-housed male mice of the BALB/c strain, and to investigate whether these effects could be extrapolated to a more aggressive mouse strain (CD-1, Parmigiani *et al.* 1999). This paper primarily deals with the housing and husbandry effects on stress-related parameters. To obtain an accurate estimate of the level of stress experienced by the mice, a wide variety of stress-related parameters were measured both during life and post-mortem, and included measures reflecting different bodily responses to challenges. As general physiological parameters, food and water intake and body weight were measured. The level of anxiety of the mice was tested in a cage emergence test. Spleen and thymus were weighed to reveal possible gross immunodepressive effects, and finally two neuro-endocrine measures reflecting HPA

axis and sympathetic activation in response to challenges (urine corticosterone levels and adrenal TH-activity, respectively) were measured. Effects on aggression are published elsewhere (Van Loo *et al.* 2003a), as is a thorough review on the housing and management of male mice (Van Loo *et al.* 2003b).

## Methods

### *Animals and husbandry*

Sixty male mice of the BALB/cAnNCrIBR (BALB/c) and sixty male mice of the Swiss:CD-1(ICR)BR (CD-1) strain were used. On arrival, all animals were 6 weeks old. Per strain, the animals were randomly divided into 20 groups of three mice, and housed in wire-topped clear Perspex Makrolon® type II cages (375 cm<sup>2</sup>, Tecniplast Milan, Italy) provided with 50 g sawdust (Lignocel® 3/4; Rettenmaier & Söhne, Ellwangen-Holzmühle, Germany). Half of the groups received nesting material (two Kleenex tissues, Kimberly-Clark Corporation®, Ede, The Netherlands) in addition to the usual bedding material ('enriched'). The other groups served as controls, without nesting material ('standard'). Pelleted food (RMH-B®, Hope Farms, Woerden, The Netherlands) and tap water were available *ad libitum*. The animal room had a controlled temperature (23–24°C), humidity (60 ± 5%) and ventilation (15–20 air changes/h). The artificial light/dark cycle was 12:12 with lights on at 07:00 h. The mice were marked on the tail as well as on the fur with a black waterproof marker to enable individual identification. Marks were renewed weekly prior to cage cleaning. After arrival the mice were allowed to adapt to their novel housing condition for one week.

### *Procedure and behavioural data collection*

Cages and wire-tops were cleaned weekly. For enriched cages, the nesting material was transferred from the dirty cage into the clean cage and half of a new tissue was added to compensate for loss due to

shredding or eating. Prior to cage cleaning, the mice were weighed and marked, wounds were counted, and food and water were weighed and refreshed. At the age of 9, 12, 15, 18 and 21 weeks, the behaviour of the mice was recorded on videotape for a period of 30 min immediately following cage cleaning. The number of agonistic encounters scored was used to classify individual animals as dominant (dom), most attacked subordinate (sub+) or least attacked subordinate (sub-). Detailed data on aggressive behaviour are published elsewhere (Van Loo *et al.* 2003a). To quantify the individual level of anxiety, mice were subjected to a cage emergence test (described by Van de Weerd *et al.* 1994) at the age of 17 weeks. In short, the cage emergence test measures the latency time for a mouse to escape from a hole in a small barren cage into a larger barren cage, with a maximum of 10 min.

#### *Urine collection and corticosterone analysis*

In order to analyse corticosterone levels, urine samples were collected at the ages of 9, 12, 15, 18 and 21 weeks. Samples were taken non-invasively 3 to 4 days after cage cleaning between 09:00 and 10:00 h (method described by Dahlborn *et al.* 1996, and modified by Van Loo *et al.* 2001). Corticosterone levels were measured using a solid phase <sup>125</sup>I radioimmunoassay (CAC<sup>®</sup> Rat Corticosterone TKRC1, Diagnostic Products Corporation, LA), and corrected for creatinine concentrations determined with the use of a commercial test combination (Creatinine, MA-KIT 10 Roche, Roche Diagnostics) on a COBAS-BIO auto-analyser (Hoffmann-La Roche BV, Mijdrecht, The Netherlands).

#### *Physiology*

At the age of 22 weeks, the mice were decapitated simultaneously per group between 09:00 and 12:00 h by three animal technicians. Spleen and thymus were dissected and weighed. Adrenals were dissected, individually shock-frozen in

5 mM Tris-HCl-buffer (pH 7.2), and stored at -70°C. Adrenal tyrosine hydroxylase activity (TH) was measured using a tyrosine-<sup>14</sup>C-assay (method described by Witte & Matthaei 1980).

#### *Statistical analysis*

Data on body weight, food and water intake, and organ weights were analysed using a general linear model for repeated measures with multiple comparisons, with age or status as a within-subject factor and strain and housing as between-subject factors. Tyrosine hydroxylase (TH) activity and Co/Cr ratio were analysed using a linear mixed effects analysis with, as fixed factors, housing (TH and Co/Cr), strain, age and status (Co/Cr); and as, random factors, group (TH) or mouse number (Co/Cr). Cage emergence time was analysed using a univariate analysis of variance with strain and housing as between-subject factors. To better conform to the normal distribution, several variables were log-transformed. When multiple comparisons were made in any of the statistical analyses, Bonferroni correction was applied (i.e. *P* value multiplied by number of comparisons, indicated by *P<sub>b</sub>*). To identify dominant and subordinate animals within each group, the level of individual aggressiveness (as observed on videotape) was used. Five out of the 40 groups showed no or hardly any aggression. As a result the hierarchies of these groups could not be reliably evaluated. When comparisons were made between dominant and subordinate mice, these groups were omitted from the analysis. All statistical tests were carried out with the aid of SPSS for MS Windows, Release 9.0 (SPSS Inc, Chicago, USA) or S-plus 2000 Professional Release 2 (© 1988–1999, MathSoft, Inc.).

## **Results**

#### *Body weight, food and water intake*

During the experiment, all mice showed a general increase in body weight (*P* < 0.001, Table 1). Enriched CD-1 mice gained more weight than CD-1 mice housed under

**Table 1** Body weight, food and water consumption (mean ± SEM) of BALB/c and CD-1 mice housed under standard or enriched conditions summarized for periods of 4 (body weight) or 5 weeks (food and water)

	Age (wk)	BALB/c		CD-1		Overall sign
		Standard	Enriched	Standard	Enriched	
Body weight (g)	7–10	20.8 ± 0.13	21.0 ± 0.13	32.9 ± 0.24	33.3 ± 0.30	A***
	11–14	23.8 ± 0.10	24.0 ± 0.09	35.8 ± 0.29	36.7 ± 0.37	S***
	15–18	25.2 ± 0.09	25.4 ± 0.09	38.3 ± 0.34	39.2 ± 0.43	SxH*
	19–22	26.3 ± 0.09	26.5 ± 0.09	39.7 ± 0.37	40.5 ± 0.45	AxS***
Food (g/group/wk)	7–11	67.3 ± 0.41	64.9 ± 0.79	95.6 ± 0.90	90.3 ± 1.23	A***
	12–16	65.6 ± 0.64	64.4 ± 0.45	91.5 ± 1.05	84.6 ± 1.13	S***
	17–21	68.4 ± 1.15	65.5 ± 0.84	91.8 ± 1.25	84.9 ± 1.32	H* AxS***
Water (ml/group/wk)	7–11	63.7 ± 0.69	62.6 ± 0.72	108.2 ± 2.06	94.2 ± 2.16	A***
	12–16	64.9 ± 0.90	64.0 ± 0.63	98.8 ± 1.74	85.3 ± 1.46	S***
	17–21	65.4 ± 0.80	64.3 ± 0.97	97.2 ± 2.18	88.7 ± 1.74	H* AxS***

A = age effect, S = strain effect, H = housing effect  
\**P* < 0.05; \*\*\**P* < 0.001

standard conditions ( $P_B < 0.05$ ). No such housing effect on weight gain was found for mice of the BALB/c strain. From the start of the experiment, CD-1 mice were significantly heavier than BALB/c mice ( $P < 0.001$ ) and gained more weight than the BALB/c mice during the experiment ( $P < 0.001$ ). Social status did not affect body weight in either strain.

An overall significant difference in food and water intake between the housing conditions was found (both  $P < 0.05$ , Table 1). Mice housed in enriched cages consumed less food and water than mice housed in standard cages. In general, food and water consumption showed a parabolic time effect. Initially, both food and water intake decreased, after which it increased again slightly ( $P < 0.001$ ). A strain difference in the total amount of food and water consumed (both  $P < 0.001$ ) and the change in food and water intake over the weeks (both  $P < 0.001$ ) was found, with CD-1 mice consuming significantly more food and water than BALB/c mice.

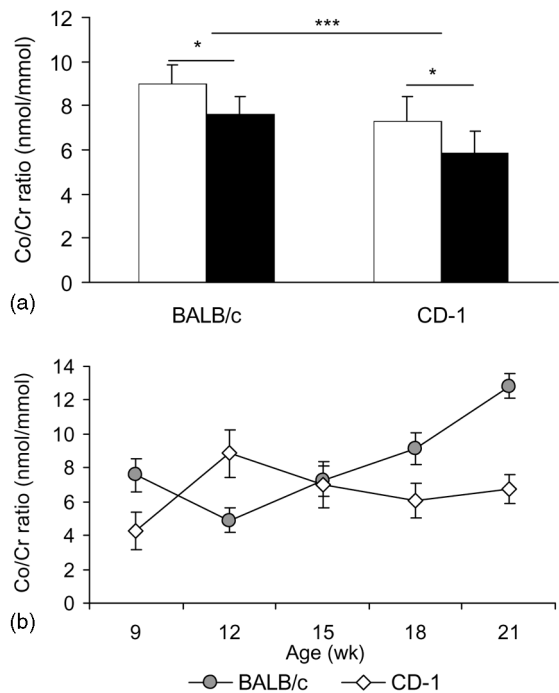
*Urine corticosterone/creatinine (Co/Cr) ratios*

For both strains, a significant housing effect on Co/Cr ratios was found (Fig 1a,  $P < 0.05$ ).

Mice housed under enriched conditions showed a lower Co/Cr ratio than mice housed under standard conditions. Furthermore, a significant strain effect was apparent. CD-1 mice showed a significantly lower Co/Cr ratio than BALB/c mice (Fig 1a,  $P < 0.001$ ). Co/Cr ratios showed a significant time effect ( $P < 0.001$ ) that differed between strains ( $P < 0.05$ , Fig 1b: pooled for housing conditions). At the age of 9 weeks, the Co/Cr ratios of the BALB/c mice were quite high, then decreased when the mice were 12 weeks old and started to show an increase again after the age of 15 weeks. The CD-1 mice, on the contrary, showed lower Co/Cr ratios at the age of 9 weeks compared to the BALB/c mice followed by an increase at the age of 12 weeks after which the Co/Cr ratios decreased slightly again. No effect of position in the dominance hierarchy on Co/Cr ratios was found.

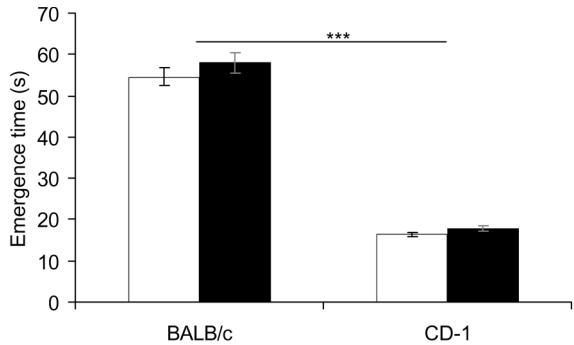
*Cage emergence test*

In the cage emergence test, again a clear strain effect was found. Mice of the BALB/c strain took significantly longer to escape from the small cage than did CD-1 mice (Fig 2,  $P < 0.001$ ). No effect of housing condition could be established.



**Fig 1** Corticosterone/creatinine ratio (mean  $\pm$  SEM) of BALB/c mice and CD-1 mice (a) housed under standard or enriched conditions, (b) at five different ages, pooled for housing conditions. \* $P < 0.05$ ; \*\*\* $P < 0.001$

□ Standard ■ Enriched



**Fig 2** Emergence time (geometric mean  $\pm$  SEM) of BALB/c mice and CD-1 mice housed under standard or enriched conditions, in the cage emergence test. \*\*\* $P < 0.001$

□ Standard ■ Enriched

*Post-mortem variables*

Thymus and spleen weight and TH activity are summarized in Table 2. The thymus weight of enriched-housed mice was significantly higher than thymus weight of standard-housed mice in both strains ( $P < 0.05$ ). Furthermore, CD-1 mice had heavier thymuses than BALB/c mice

( $P < 0.05$ ). In contrast, spleen weight of CD-1 mice was significantly lower than spleen weight of BALB/c mice ( $P < 0.05$ ), and a significant interaction between strain and housing condition was found ( $P < 0.01$ ). For the enriched-housed mice, spleen weight did not differ between the strains, while in the standard housing BALB/c mice showed

**Table 2** Organ weights (mg), TH activity (nmol/h/adrenal pair); mean ± SEM

Parameter		BALB/c		CD-1	
		Standard	Enriched	Standard	Enriched
Thymus		30.00 ± 0.90 <sup>a</sup>	32.30 ± 1.30 <sup>b</sup>	33.30 ± 1.70 <sup>c</sup>	35.70 ± 1.70 <sup>d</sup>
Spleen		108.70 ± 5.30 <sup>e</sup>	100.30 ± 4.50	92.30 ± 3.50 <sup>f</sup>	99.70 ± 3.60
TH	dom <sup>g</sup>	8.45 ± 1.10	8.33 ± 1.79	8.38 ± 1.21	8.44 ± 0.87
	sub+	8.46 ± 1.03	8.21 ± 2.03	7.19 ± 0.90	6.87 ± 1.20
	sub- <sup>h</sup>	7.19 ± 0.73	6.79 ± 0.73	6.95 ± 0.61	7.30 ± 1.16
	Unknown status	8.28 ± 1.14	4.40 ± 0.54	–	5.18 ± 0.99

a/b – c/d, a/c – b/d:  $P < 0.05$

e–f:  $P_{\text{B}} < 0.05$

g–h:  $P_{\text{B}} < 0.1$

a heavier spleen weight compared to the CD-1 mice ( $P_{\text{B}} < 0.05$ ).

The TH activity tended to differ for individuals with different positions in the dominance hierarchy (Table 2,  $P < 0.1$ ), and no effects of housing condition or strain were found. Multiple comparisons, pooled for housing and strain, revealed that dominant mice tended to have higher TH activity than least attacked subordinate mice ( $P_{\text{B}} < 0.1$ ), while most attacked subordinate mice were intermediate.

**Discussion**

*Housing condition effects*

Housing condition affected several physiological parameters, i.e. food and water intake, body weight, corticosterone levels and thymus weight. Food and water intake for mice housed under enriched conditions were lower than for mice housed under standard conditions while body weight of enriched-housed CD-1 mice was higher than for standard-housed CD-1 mice, and BALB/c mice of different housing conditions gained equal weight. This is in accordance with Dahlborn *et al.* (1996), Van de Weerd *et al.* (1997) and Van Loo *et al.* (2002), who found that mice from cages enriched with nesting material gained equal or more weight than mice from standard housing conditions, although they consumed less food. It was hypothesized that nesting material allows the mice to regulate their body temperature and, as a consequence, might decrease the

need for food and water. Others stipulate that laboratory animals kept in standard conditions eat and drink more than animals housed in enriched cages due to boredom (Fiala *et al.* 1977, Van de Weerd *et al.* 1994). Excessive feeding and drinking have been reported as behavioural reactions to prolonged encagement (Wemelsfelder 1993). A discrepancy between food and water intake and body weight gain may also be related to the amount of stress experienced. Many reports have shown that chronic stress can produce a decrease in body weight, or a reduced weight gain in animals that are still growing (Manser 1992).

Corticosterone levels were higher and thymus weight was lower for standard-housed mice compared to mice housed with nesting material that was transferred during cage cleaning. An increase in baseline levels of corticosterone may be an indicator of chronic stress (Manser 1992, Shepherdson *et al.* 1998), and a decreased thymus weight is consistent with higher baseline corticosterone levels (Manser 1992, Moberg & Mench 2000). The lower corticosterone levels of enriched-housed mice found in this experiment are contrary to the results of Haemisch and Gärtner (1994). They found that enriched-housed mice showed increased levels of corticosterone, which they explained by their finding that mice in enriched cages were more aggressive and failed to maintain stable dominance relationships. An important difference between the latter and this experiment is the type of enrichment used. In a previous study

(Van Loo *et al.* 2002) inter-male aggression and corticosterone levels increased in mice housed in cages, structured with a shelter, comparable to the enrichment used by Haemisch & Gärtner (1994), while inter-male aggression decreased in mice housed with nesting material. In another experiment (Van Loo *et al.* 2000) the transfer of nesting material during cage cleaning clearly decreased aggression between male mice. Besides the transfer of familiar odours that may have reduced stress, the provided nesting material (tissues) itself could be used to hide from other mice besides being manipulated for nest building, which gave the mice the possibility of having some control over their environment. Controllability of the environment, next to predictability, has been reported to be an important factor influencing the amount of stress experienced by animals in an environment (Weiss 1972, Manser 1992, Wiepkema & Koolhaas 1993, Shepherdson *et al.* 1998). Moreover, preference tests have shown that both mice and rats clearly prefer nesting material to rigid structures such as a platform, a nest box (Bradshaw & Poling 1991, Van de Weerd *et al.* 1998, Van Loo submitted) or a shelter (unpublished data).

#### *Strain, social status and age effects*

Several parameters indicated that BALB/c mice may be more susceptible to social stress than CD-1 mice. BALB/c mice had higher urine corticosterone levels and they were considerably slower to escape in the cage emergence test, indicating more anxiety. In accordance with this, Kopp *et al.* (1999) showed that mice of the BALB/c strain are particularly susceptible to chronic stress exposure compared to several other inbred mouse strains. Although BALB/c mice had lower body weights with accordingly lighter organs, the spleen weight of BALB/c mice was significantly heavier than that of CD-1 mice. The spleen reacts actively to blood-borne antigens, and would thus be expected to increase in weight when mice are wounded (Roitt 1988). Although CD-1 mice showed more aggressive interactions, BALB/c mice were

generally more wounded (Van Loo *et al.* 2003a).

Corticosterone levels of BALB/c mice followed a time curve similar to time curves found in previous and other experiments (Bronson 1973, Goldsmith *et al.* 1978, Van Loo *et al.* 2001, 2002): after grouping, levels were quite high due to the social tension associated with establishment of a stable hierarchy. Levels then decreased as the hierarchy within groups remained stable and thereafter started to rise again, paralleling an increase in aggression as the mice became older (Van Loo *et al.* 2003a). For CD-1 mice, on the other hand, corticosterone levels increased from the age of 9 to 12 weeks and declined slightly afterwards. A reason for this is difficult to allege.

The correlation between social status and both corticosterone levels and TH activity were investigated as well. These measures reflect the HPA axis and sympathetic activation in response to challenges, respectively (Manser 1992, Moberg & Mench 2000). For corticosterone levels, no effect of social status could be revealed, although Co/Cr ratios of dominant and most-attacked subordinate mice significantly correlated to the level of aggression (Van Loo *et al.* 2003a). This may indicate that the level of aggression within a group influences corticosterone levels to a greater extent than position in the hierarchy. In general, the TH activity of the dominant mice tended to be higher than for the least attacked subordinate mice, while the TH activity of most attacked subordinate mice was intermediate. Previous findings and those of others (Haemisch & Gärtner 1996, Van Loo *et al.* 2001, 2002) are in agreement with these results. The most obvious explanation being that both maintaining dominance and being defeated is stressful, while accepting a subordinate status without ever challenging the dominant male may be less stressful (Busser *et al.* 1974).

## **Conclusion and recommendations**

Long-term enrichment with nesting material combined with the repeated transfer of



nesting material when cleaning the cages influenced several stress-related parameters. The corticosterone levels of enriched-housed mice were lower, their thymus weight was increased, and they consumed less food and water than standard-housed mice while gaining more or equal weight. Since these results are an indication for reduced levels of stress in enriched-housed conditions, the provision of nesting material combined with its transfer during cage cleaning is recommended for group-housed male laboratory mice.

**Acknowledgments** The authors would like to thank T. Blankensteijn, I. Lemmens and J. Wolfswinkel for assistance with laboratory analyses and K. Brandt, S. Versteeg, P. Rooymans and R. Timmermans for technical assistance with euthanasia and dissection.

## References

- Ambrose N, Morton DB (2000) The use of cage enrichment to reduce male mouse aggression. *Journal of Applied Animal Welfare Sciences* **3**, 117–25
- Armstrong KR, Clark TR, Peterson MR (1998) Use of corn-husk nesting material to reduce aggression in caged mice. *Contemporary Topics in Laboratory Animal Science* **37**, 64–6
- Bradshaw AL, Poling A (1991) Choice by rats for enriched versus standard home cages: plastic pipes, wood platforms, wood chips and paper towels as enrichment items. *Journal of Experimental Analysis of Behaviour* **55**, 245–50
- Baumans V (2000) Environmental enrichment: a right for rodents! In: *Progress in the Reduction, Refinement and Replacement of Animal Experimentation* (Balls M, Van Zeller EM, Halder ME, eds). Amsterdam: Elsevier, pp 1251–5
- Bronson FH (1973) Establishment of social rank among grouped male mice: relative effects on circulating FSH, LH, and corticosterone. *Physiology & Behavior* **10**, 947–51
- Busser J, Zweep A, Van Oortmerssen GA (1974) Variability in the aggressive behaviour of *Mus musculus* domesticus, its possible role in population structure. In: *The Genetics of Behaviour* (Van Abeelen JNE, ed). Amsterdam: North-Holland Publishing Company, pp 185–99
- Chamove AS (1989) Environmental enrichment: a review. *Animal Technology* **40**, 155–78
- Cooper JJ, Nicol CJ (1996) Stereotypic behaviour in wild caught and laboratory bred bank voles (*Clethrionomys glareolus*). *Animal Welfare* **5**, 245–57
- Council of Europe (1997) *Resolution on the Accommodation and Care of Laboratory Animals*, adopted by the Multilateral Consultation on 30 May 1997
- Dahlborn K, Van Gils BAA, Van de Weerd HA, Van Dijk JE, Baumans V (1996) Evaluation of long-term environmental enrichment in the mouse. *Scandinavian Journal of Laboratory Animal Science* **1**, 97–106
- Dean SW (1999) Environmental enrichment of laboratory animals used in regulatory toxicology studies. *Laboratory Animals* **33**, 309–27
- Eskola S, Kaliste-Korhonen E (1999) Aspen wood-wool is preferred as a resting place but does not affect intracage fighting of male BALB/c and C57BL/6J mice. *Laboratory Animals* **33**, 108–21
- Fiala B, Snow F, Greenough W (1977) Impoverished rats weigh more than enriched rats because they eat more. *Developmental Psychobiology* **10**, 537–41
- Goldsmith JF, Brain PF, Benton D (1978) Effects of the duration of individual or group housing on behavioural and adrenocortical reactivity in male mice. *Physiology & Behavior* **21**, 757–60
- Haemisch A, Gärtner K (1994) The cage design affects intermale aggression in small groups of male laboratory mice: strain specific consequences on social organization, and endocrine activations in two inbred strains (DBA/2J and CBA/J). *Journal of Experimental Animal Science* **36**, 101–16
- Haemisch A, Voss T, Gärtner K (1994) Effects of environmental enrichment on aggressive behavior, dominance hierarchies, and endocrine states in male DBA/2J mice. *Physiology & Behavior* **56**, 1041–8
- Haemisch A, Gärtner K (1996) Dissociation between adrenal tyrosinehydroxylase and phenylethanolamine-N-methyltransferase activities following repeated experience of defeats in individually housed male DBA/2J mice. *Physiology & Behavior* **59**, 1117–22
- Kopp C, Vogel E, Misslin R (1999) Comparative study of emotional behaviour in three inbred strains of mice. *Behavioural Processes* **47**, 161–74
- Manser CE (1992) *The Assessment of Stress in Laboratory Animals*. West Sussex: RSPCA
- McGregor PK, Ayling SJ (1990) Varied cages result in more aggression in male CFLP mice. *Applied Animal Behaviour Science* **26**, 277–81
- McGregor PK, Barnard C, Hurst JL (1991) Reply to Jones RB 1991: Varied cages and aggression. *Applied Animal Behavioural Science* **27**, 297–9
- Moberg GP, Mench JA (2000) *The Biology of Animal Stress. Basic Principles and Implications for Animal Welfare*. Wallingford: CABI Publishing
- Newberry RC (1995) Environmental enrichment: increasing the biological relevance of captive environments. *Applied Animal Behaviour Science* **44**, 229–43
- Parmigiani S, Palanza P, Rodgers J, Ferrari PF (1999) Selection, evolution of behavior and animal mod-

- els in behavioral neuroscience. *Neuroscience and Biobehavioral Reviews* **23**, 957–70
- Roitt I (1988) *Essential Immunology*, 6th edn. Oxford: Blackwell Scientific Publications
- Shepherdson DJ, Mellen JD, Hutchins M (1998) *Second Nature, Environmental Enrichment for Captive Animals*. London: Smithsonian Institution Press
- Van Loo PLP, Kruitwagen CLJJ, Van Zutphen LFM, Koolhaas JM, Baumans V (2000) Modulation of aggression in male mice: influence of cage cleaning regime and scent marks. *Animal Welfare* **9**, 281–95
- Van Loo PLP, Mol JA, Koolhaas JM, Van Zutphen LFM, Baumans V (2001) Modulation of aggression in male mice: influence of group size and cage cleaning. *Physiology & Behavior* **72**, 675–83
- Van Loo PLP, Kruitwagen CLJJ, Koolhaas JM, Van de Weerd HA, Van Zutphen LFM, Baumans V (2002) Influence of cage enrichment on aggressive behaviour and physiological parameters in male mice. *Applied Animal Behaviour Science* **76**, 65–81
- Van Loo PLP, Van der Meer E, Kruitwagen CLJJ, Koolhaas JM, Van Zutphen LFM, Baumans V (2003a) Strain-specific aggressive behaviour of male mice submitted to different husbandry procedures. *Aggressive Behavior* **29**, 69–80
- Van Loo PLP, Van Zutphen LFM, Baumans V (2003b) Male management: coping with aggression problems in male laboratory mice. *Laboratory Animals* **37**, 300–13
- Van Loo PLP, Blom HJM, Meijer MK, Baumans V (submitted) Ask the Animal! The use of commercially available environmental enrichment by laboratory mice
- Van de Weerd HA, Baumans V, Koolhaas JM, Van Zutphen LFM (1994) Strain specific behavioural response to environmental enrichment in the mouse. *Journal of Experimental Animal Science* **36**, 117–27
- Van de Weerd HA, Van Loo PLP, Van Zutphen LFM, Koolhaas JM, Baumans V (1997) Nesting material as environmental enrichment has no adverse effect on behavior and physiology of laboratory mice. *Physiology & Behavior* **62**, 1019–28
- Van de Weerd HA, Van Loo PLP, Van Zutphen LFM, Koolhaas JM, Baumans V (1998) Strength of preference for nesting material as environmental enrichment for laboratory mice. *Applied Animal Behaviour Science* **55**, 369–82
- Ward GE, Fiat RA, DeMille D (1991) Environmental enrichment for laboratory mice. *Animal Technology* **42**, 149–56
- Weiss JM (1972) Psychological factors in stress and disease. *Scientific American* **226**, 104–13
- Wemelsfelder F (1993) *Animal Boredom. Towards an Empirical Approach of Animal Subjectivity* (PhD Thesis). Utrecht: Elinkwijk
- Wiepkema PR, Koolhaas JM (1993) Stress and animal welfare. *Animal Welfare* **2**, 195–218
- Witte PU, Matthaei H (1980) *Mikrochemische Methoden für neurobiologische Untersuchungen*. Berlin: Springer
- Würbel H, Chapman R, Rutland C (1998) Effect of feed and environmental enrichment on development of stereotypic wire-gnawing in laboratory mice. *Applied Animal Behaviour Science* **60**, 69–81